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Effect of Temperature and Relative Humidity on the Survival of Foodborne Viruses during Food Storage

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Millions of people suffer from foodborne diseases throughout the world every year, and the importance of food safety has grown worldwide in recent years. The aim of this study was to investigate the survival of hepatitis A virus (HAV) and viral surrogates of human norovirus (HuNoV) (bacteriophage MS2 and murine norovirus [MNV]) in food over time. HAV, MNV, and MS2 were inoculated onto either the digestive gland of oysters or the surface of fresh peppers, and their survival on these food matrices was measured under various temperature (4°C, 15°C, 25°C, and 40°C) and relative humidity (RH) (50% and 70%) conditions. Inoculated viruses were recovered from food samples and quantified by a plaque assay at predetermined time points over 2 weeks (0, 1, 3, 7, 10, and 14 days). Virus survival was influenced primarily by temperature. On peppers at 40°C and at 50% RH, >4- and 6-log reductions of MNV and HAV, respectively, occurred within 1 day. All three viruses survived better on oysters. In addition, HAV survived better at 70% RH than at 50% RH. The survival data for HAV, MS2, and MNV were fit to three different mathematical models (linear, Weibull, and biphasic models). Among them, the biphasic model was optimum in terms of goodness of fit. The results of this study suggest that major foodborne viruses such as HAV and HuNoV can survive over prolonged periods of time with a limited reduction in numbers. Because a persistence of foodborne virus on contaminated foods was observed, precautionary preventive measures should be performed.

Throughout the world, millions of people suffer from foodborne diseases every year, and the number of patients is predicted to increase in proportion to global warming (1–3). Foodborne diseases are transmitted via fecal-oral routes and person-to-person contact. Among the numerous enteric pathogenic viruses, human norovirus (HuNoV) and hepatitis A virus (HAV) are considered to be the most important. In recent reports (4–6), foodborne outbreaks of HuNoV and HAV were closely associated with fresh produce (e.g., leafy greens and fruits, etc.), shellfish (oysters and clams, etc.), and ready-to-eat foods (e.g., salads and sandwiches, etc.).

HuNoV is considered to be the leading cause of foodborne outbreaks worldwide (7). However, despite its importance to public health, the inability to cultivate HuNoV *in vitro* makes research difficult (8, 9). Therefore, several viruses, including feline calicivirus (FCV), murine norovirus (MNV), Tulane virus, and bacteriophage MS2, have been proposed as surrogates for HuNoV (10–13) due to similarities in size and genome structure. The incidence rate of hepatitis A has decreased in most developed countries, but massive sporadic outbreaks of hepatitis A from contaminated foods have been continuously reported worldwide (14–16). For example, >2 million patients were infected through the consumption of HAV-contaminated oysters in China (17).

Viruses cannot replicate in food or water because an appropriate host is required for viral replication. After contamination occurs, the virus is subject to decay. Therefore, the survival of foodborne viruses depends on various factors, such as the stability of the virus, treatment of the food, and environmental conditions (18). Enteric viruses were reported to be relatively resistant to various environmental factors (e.g., low pH, heat, and water activity) (19), and viral persistence in various environments (e.g., marine and soil environments) and fresh foods has been reported (20, 21).

Some of the most commonly identified contaminated food items are oysters and fresh vegetables. In particular, oysters are known to be one of the important vehicles involved in both HuNoV and HAV outbreaks (22–24). Contamination of oysters readily occurs as they bioconcentrate waterborne viruses from filter feeding in fecally contaminated waters (25–27). Consequentially, the consumption of contaminated raw or uncooked oysters increases the chance of a foodborne outbreak. Quick-frozen oysters, which are sucked, packaged, and frozen to prolong their shelf-life, have been widely distributed in the market. Previous studies reported that imported frozen oysters were attributed to outbreaks of HuNoV gastroenteritis in Australia (28) and the United States (29). This suggests that frozen oysters can also be associated with foodborne outbreaks and brings concerns with expanding international trade. The demand for fruits and raw vegetables has markedly increased in recent years due to growing interest in healthy food. Potential routes of contamination of these food items include soil, irrigation water, inadequately composted fertilizer, and food handlers' poor hygiene (30). Peppers are steadily consumed vegetables (31) and popular ingredients in

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fresh-cut salads. However, little is known about the survival of viruses on peppers under various environmental conditions. Although they have not been directly linked to viral outbreaks, the increasing demand for fresh produce leaves peppers as a potential vector for a foodborne outbreak.

In this study, we investigated the survival of HAV and two surrogates of HuNoV, MS2 and MNV, under various temperature and relative humidity (RH) conditions. Two typical foods eaten raw (i.e., oyster and pepper) were selected to be materials in this experiment. Viral survival on either the digestive gland of oysters or the surface of peppers was characterized. For further analysis, the survival results for the tested viruses under various environmental conditions were fit to three different mathematical models, and *D* (days to 1-log reduction) values were evaluated by using the best-fit model.

MATERIALS AND METHODS

Preparation of viral stocks. HAV and MNV were cultivated according to methods described previously (32). Briefly, HAV and MNV were propagated in FRhK-4 cells and RAW 264.7 cells, respectively, in Dulbecco's modified Eagle's medium (DMEM) (Gibco, Grand Island, NY, USA) supplemented with 10% heat-inactivated fetal bovine serum (Gibco), 10 mM HEPES (Gibco), 10 mM sodium bicarbonate (Gibco), 10 mM minimum essential medium nonessential amino acids (Gibco), and gentamicin (50 g/liter) (Gibco). FRhK-4 cells were grown to confluence and then infected with HAV for 1 h. After 9 to 10 days of incubation at 37°C in 5% CO_2, HAV-infected cells were treated with three freeze-thaw cycles and purified with chloroform (Amresco, Solon, OH, USA), followed by centrifugation at 5,000 × *g* for 20 min at 4°C. To concentrate HAV, supernatants were collected and centrifuged by using an ultrafilter (Amicon Ultra-15; Millipore, Billerica, MA, USA) at 5,000 × *g* for 20 min at 4°C 15 to 20 times. Virus-containing supernatants were collected in 0.1-ml aliquots and stored at −70°C. MNV was inoculated in confluent RAW 264.7 cells for 1 h and then incubated for 4 to 5 days. MNV-infected cells were exposed to three free-thaw cycles, mixed with an equal volume of chloroform (Amresco), and then centrifuged at 5,000 × *g* for 20 min at 4°C. Supernatants were subjected to ultrafiltration at 5,000 × *g* for 20 min at 4°C 15 to 20 times. Subsequently, virus-containing supernatants were kept frozen at −70°C until use. Bacteriophage MS2 was propagated in *Escherichia coli* C3000. MS2 stocks (0.1 ml) were thoroughly mixed with 0.3 ml of subcultured *E. coli* C3000 cells and 30 ml of molten tryptic soy agar (TSA) (BD, Sparks, MD, USA), and the mixtures were then poured into plates. After incubation overnight at 37°C, the surfaces of the agar plates were washed several times with phosphate-buffered saline (PBS) to collect the virus. The MS2-containing suspension was collected in 0.1-ml aliquots and stored at −70°C until use. The titers of virus stocks were quantitated by a plaque assay.

Characterization of viral survival on oysters and peppers under various environmental conditions. Fresh peppers (*Capsicum annuum*) were purchased from the local grocery market before the start of each experiment. Frozen oysters (*Crassostrea gigas*) (shucked) were bought in bulk from the local fish market in advance and stored at −70°C until use. We decided to use frozen oysters with consideration of their wide distribution in the market. Peppers were washed with running water and then cut into 2-cm squares to be similar to fresh-cut vegetables in the market. Frozen oysters were defrosted at room temperature and then rinsed with running water. Oysters possessing an intact midgut gland were selected as final samples for inoculation. Both peppers and oysters were placed into petri dishes and disinfected with UV radiation for 30 min. After preparation of the food samples, 100 μl of HAV (2×10^6 PFU/ml), MS2 (2×10^7 PFU/ml), or MNV (2×10^7 PFU/ml) was inoculated onto the smooth surface of the pepper samples. For the oysters, to minimize weight differences between samples, we determined that each single sample was composed of two oysters. To inoculate 100 μl of virus per sample, 50 μl of virus was directly injected into a piece of the midgut gland on each oyster by using a disposable syringe. The midgut gland, called the region of viral bioaccumulation, was dissected from the whole oyster and immersed in 20 ml of PBS. Virus-inoculated food samples were placed into a temperature- and RH-controlled environmental chamber (TH-TG-300; Jeio Tech, Daejeon, South Korea). To simulate various storage conditions, four different temperature settings (4°C, 15°C, 25°C, and 40°C) and two different RH settings (50% and 70%) were used. While infected food samples were stored in the chamber, viruses were recovered from samples at six time points (0, 1, 3, 7, 10, and 14 days). On the day of inoculation (day 0), viruses were recovered immediately after the inoculation step and then used as a control for viral reduction. Viruses inoculated onto either peppers or oysters were recovered by using sonication or centrifugation methods, respectively. Briefly, peppers were immersed in 20 ml PBS and sonicated for 30 min at 4°C. For oysters, a set of midgut tissues was placed into a filter bag with 20 ml PBS and homogenized by using a stomacher (Seward, West Sussex, United Kingdom) for 30 s. After homogenization, 15 ml of the homogenate was transferred into a conical tube, and centrifugation was performed at 10,000 × *g* for 30 min at 4°C. Viruses recovered from supernatants were analyzed by a plaque assay. When the virus titer was below the limit of detection (LOD), the experiment was repeated by using shorter recovery times (6, 12, and 24 h). The experiment was not performed with MNV at 70% RH due to mold contamination. All experiments were performed in triplicate.

Quantification of infectious viruses by a plaque assay. Infectious viral particles were measured by plaque assay (HAV and MNV) and by single-agar-layer (SAL) (MS2) methods. To quantify HAV or MNV, confluent cells (FRhK cells for HAV or RAW 264.7 cells for MNV) were cultured in 6-well plates. Viral suspensions recovered from food samples were serially diluted in serum-free DMEM from 10^{-1} to 10^{-5}. The cells were inoculated with 500 μl of recovered virus for 1 h and then covered with 1.5% SeaPlaque agarose (Lonza, Rockland, ME, USA) mixed with an equal volume of plaque assay medium. MNV plaques were counted within 3 to 5 days. Plates containing HAV were incubated for 7 days at 37°C in 5% CO_2, followed by a second overlay of agarose containing a 1% neutral red solution (Sigma-Aldrich, St. Louis, MO, USA). The virus was incubated for another 3 days, and plaques were then counted. To quantify MS2, the SAL method was used, according to U.S. Environmental Protection Agency (EPA) standard protocols (33). Serial 10-fold dilutions of MS2 suspensions recovered from food samples were mixed with *E. coli* C3000 cells and TSA, and the mixtures were poured into 150-mm petri dishes. After overnight incubation at 37°C, counts were obtained from plates containing between 3 and 300 plaques.

Fitting the experimental data to three different models. To predict the survivability of viruses based on time (days) after inoculation, modeling analysis was performed. We compared three survival models (linear, Weibull, and biphasic models) to find the model that best fit our observed survival data. Survival curves were fit to the observed data points by using SigmaPlot version 10.0 (Systat Software, San Jose, CA, USA), and predictive curves were overlaid upon the survival curves by using the formula for each model. Finally, the goodness of fit of the models was evaluated by comparing parameters such as the regression coefficient (R^2), root mean square error (RMSE), and Akaike information criterion (AIC) (34). The three models described below were used for this study.

The linear model is a general model for explaining the inactivation of microorganisms at a constant temperature over time (35). The equation for the linear model is $\log_{10} N_t/N_0 = -t/D$, where N_0 and N_t denote the initial virus titer (PFU/ml) and the virus titer after an elapsed time (PFU/ml), respectively; t is exposure time (days); and D is the D value (decimal reduction time), which is the time required to inactivate 90% of the virus, indicating the thermal resistance of a microorganism (36).

Microbial survival curves may be nonlinear, having a sigmoidal shape with a shoulder and tailing. The Weibull distribution is a well-known nonlinear model with the equation $\log_{10} N_t/N_0 = -bt^n$ (36, 37), where t is exposure time (days) and b and n represent the scale parameter and shape

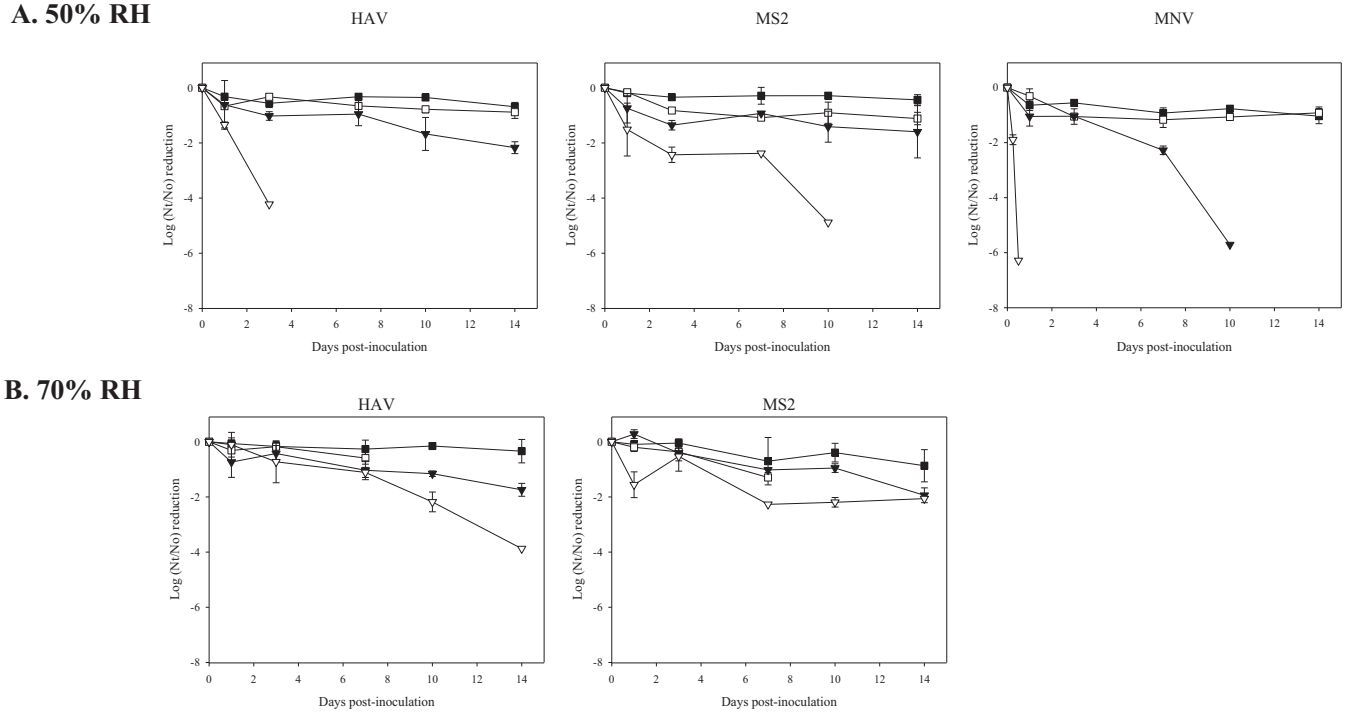

FIG 1 Inactivation curves of foodborne viruses on oysters at 50% RH (A) and 70% RH (B), determined by a plaque assay. Each graph contains four survival curves at the following four temperatures: 4°C (■), 15°C (□), 25°C (▼), and 40°C (▽). The error bars indicate standard deviations. There are no data for MNV at 70% RH due to mold contamination. In the cases of HAV and MS2, mold appeared on foods after 10 days at 70% RH (B) and at 15°C (□), at which point the experiments were stopped.

parameter, respectively. The main advantage of the Weibull model is that is describes both downward concave (shoulder) survival curves ($n > 1$) and upward concave (tailing) survival curves ($n < 1$).

The biphasic model proposed by Kamau et al. (38) describes the inactivation of microorganisms in two fractions. This model applies a logistic equation to fit linear and nonlinear survival curves. The model also assumes that microorganism numbers decrease exponentially throughout the two phases and that the rate of decline of each phase is independent. The equation of biphasic model is as follows:

$$\log_{10}\frac{N(t)}{N_0} = \log_{10}\left(\frac{2f}{1+e^{b1t}} + \frac{2(1-f)}{1+e^{b2t}}\right)$$

where f represents first fraction on the survival curve, b_1 is the specific death rate in the first slope, $(1 - f)$ denotes the second fraction on the survival slope, b_2 denotes the specific death rate in the second slope, and t is the exposure time (days) (39).

Statistical analysis. Means and standard deviations of the data obtained were calculated by using Microsoft Excel 2010 (Microsoft Corporation, USA). Statistical significance was analyzed by one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test, using SPSS Statistics version 21.0 (IBM, NY, USA). A P value of <0.05 was considered statistically significant.

RESULTS

Inactivation curves of viruses under different temperature and RH conditions. The survival kinetics of HAV, MS2, and MNV on oysters and peppers under different temperature and RH conditions are described in Fig. 1 and 2, respectively. Regardless of the RH or the inoculated food, all tested viruses survived best at the lowest temperature (4°C) and were inactivated most at the highest temperature (40°C). On oysters, a <1-log reduction of both HAV and MNV occurred at 4°C, even after 14 days. However, a >5-log reduction of MNV occurred on peppers at 4°C. MNV showed the shortest survival duration on peppers at all temperatures compared to the other viruses. Viral survival was better on oysters than on peppers, regardless of the temperature conditions; all tested viruses, particularly MNV, survived much better on oysters. At a given temperature, HAV survived better at higher RH, while MS2 survived better at lower RH. At 40°C, inactivation of HAV was >1 log at 50% RH but only 0.1 log at 70% RH at 1 day postinoculation.

Model evaluation of the experimental data. To assess the survivability of each virus, three different models were applied to the observed data: (i) a linear exponential model, (ii) the Weibull model as a nonlinear model, and (iii) the biphasic model as another nonlinear model. The goodness of fit of each model was evaluated based on R^2, RMSE, and AIC, and the results are summarized in Table 1 (for details, see Tables S1 to S3 in the supplemental material). R^2 and RMSE are the correlation coefficient and the average deviation between the observed and predicted data, respectively. Generally, higher R^2 and lower RMSE values indicate a better-fitting model (35). Under all environmental conditions and for both food types, the linear model showed the poorest fit in terms of both R^2 (0.67) and RMSE (0.55). In contrast, there were slight differences between the Weibull and biphasic models. The mean R^2 values for each virus were 0.89 (HAV), 0.93 (MS2), and 0.92 (MNV) for the Weibull model and 0.94 (HAV), 0.96 (MS2), and 0.99 (MNV) for the biphasic model. The biphasic model gave a lower mean RMSE (0.22) than did the Weibull model (0.37). As the models include different numbers of parameters, AIC based on the maximized likelihood was implemented, where lower val-

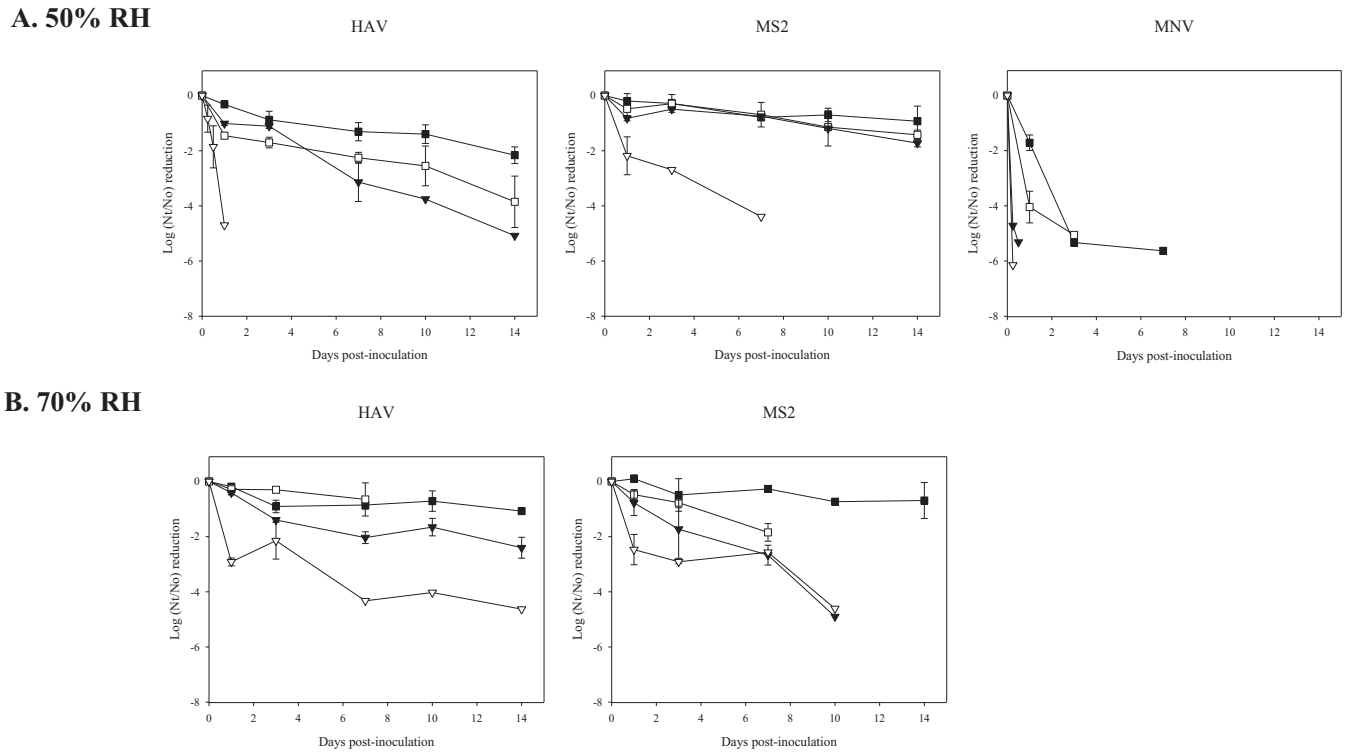

FIG 2 Inactivation curves of foodborne viruses on peppers at 50% RH (A) and 70% RH (B), determined by a plaque assay. Each graph contains four survival curves at the following four temperatures: 4°C (■), 15°C (□), 25°C (▼), and 40°C (▽). The error bars indicate standard deviations. There are no data for MNV at 70% RH due to mold contamination. In the cases of HAV and MS2, mold appeared on foods after 10 days at 70% RH (B) and at 15°C (□), at which point the experiments were stopped.

ues indicate a better fit to the survival curves. Compared with the Weibull model and the linear model, the biphasic model showed low values of AIC overall.

To further evaluate the goodness of fit of the Weibull model and the biphasic model, the correlations between observed and predicted data were compared (Fig. 3). The plot indicates that the biphasic model predicted the data better than did the Weibull model. As the biphasic model appeared to be the best model for explaining our data, we used this model for further analysis. Table S4 in the supplemental material summarizes the parameters of the fitted biphasic model. Table 2 shows the *D* values of the three tested viruses predicted by the biphasic model under different temperature and RH conditions.

DISCUSSION

In this study, we characterized the survival of HAV, MS2, and MNV on either oysters or vegetables under various environmental conditions and fit the data to three commonly used inactivation models (linear, Weibull, and biphasic models). Our study indicated that both temperature and food type are major factors influencing inactivation of all of the tested viruses. For example, at 4°C, viruses inoculated onto oysters were predicted to show only 1-log inactivation after 29 days (HAV), 48 days (MS2), and 14 days (MNV) postinoculation. The strong persistence of HAV and MNV on oysters has been reported previously (21, 40, 41). Our data support the current epidemiology suggesting that consumption of virus-contaminated oysters causes gastroenteritis worldwide. In addition, HAV and MS2 on peppers were observed to remain infectious until 14 days postinoculation at 4°C and 15°C. According to the predicted *D* value, a maximum of 17 days was required for a 1-log reduction of HAV or MS2 at 4°C. However, <1 day was required for MNV. The longer survival times at lower temperatures shown by our data are consistent with data reported in previous studies. Sun et al. (4) investigated the survival of HAV on the surface of green onions at temperatures ranging from 3°C to 24°C. When HAV-contaminated green onions were stored at 3°C, *D* values were 29 to 30 days, indicating even greater survivability than that shown by our data. In addition, Croci et al. (42) reported that HAV on lettuce remained viable for over 9 days, and the virus was completely inactivated on carrots on day 4 and on fennel on day 7. While it is not possible to make a direct comparison with data from previous studies due to differences in experimental conditions, the results are similar, demonstrating that lower-temperature conditions decrease the reduction of viruses on foods. Considering that vegetables and shellfish are typically

TABLE 1 Summary of estimated parameters for fitting the biphasic, Weibull, and linear models to virus experimental data[a]

	Biphasic distribution			Weibull distribution			Linear distribution		
Virus	R^2	RMSE	AIC	R^2	RMSE	AIC	R^2	RMSE	AIC
HAV	0.93	0.20	−8.81	0.89	0.21	−6.24	0.69	0.36	−3.42
MS2	0.91	0.29	−6.24	0.88	0.33	−5.19	0.68	0.43	−2.93
MNV	0.99	0.18	−82.86	0.92	0.57	0.73	0.65	0.86	4.65

[a] R^2 is the correlation coefficient between the predicted and observed data, RMSE is the root mean square error, and AIC is the Akaike information criterion.

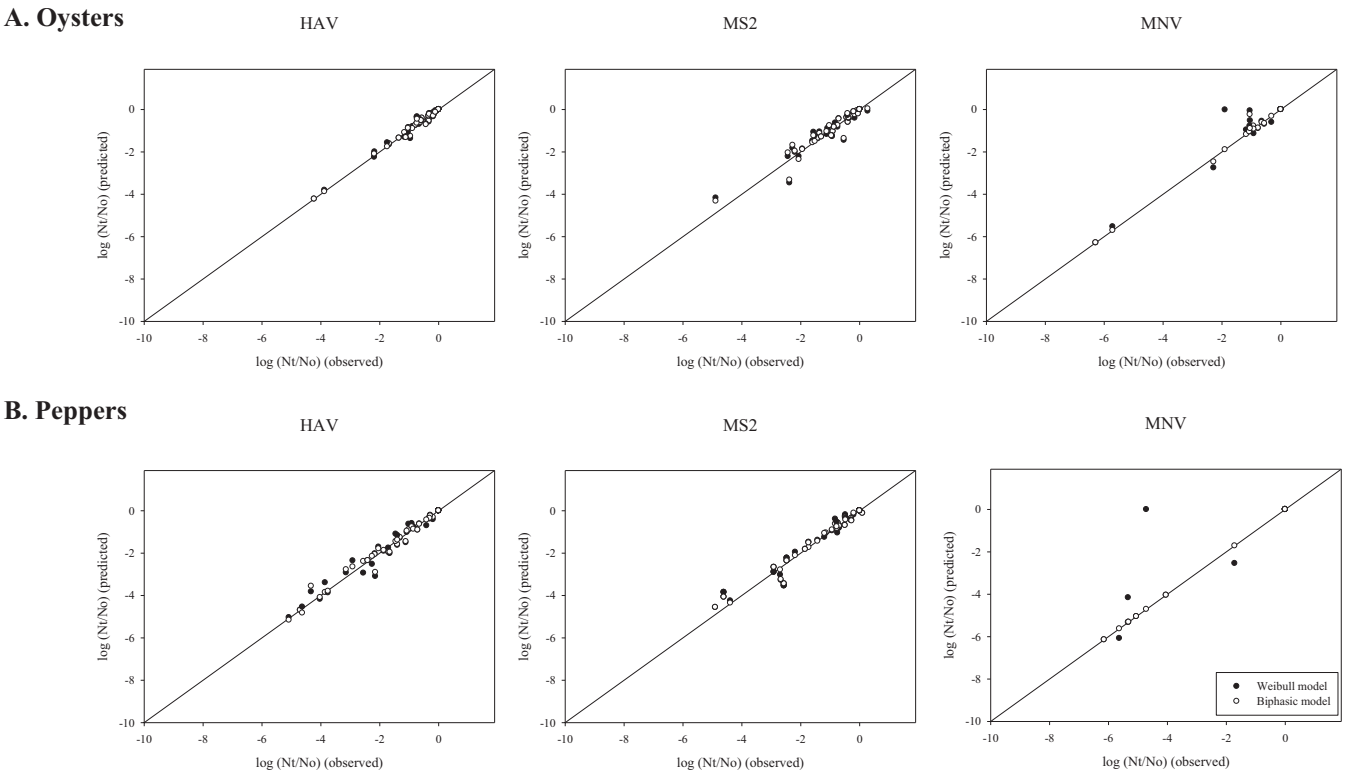

FIG 3 Correlation between the observed and predicted log reductions of foodborne viruses on oysters (A) and peppers (B). Circles deviating from the line of equivalence indicate discrepancies between the observed and predicted values.

stored under refrigerated conditions, the survival properties of foodborne viruses at 4°C are remarkably important. In addition, under most conditions, infectious viruses were still detectable after 2 weeks.

In general, viruses survived better in oysters than on peppers in this study. In addition, inactivation rates of viruses, particularly MNV, were different between oysters and peppers. Given the same quantity of inoculated virus, at 25°C, MNV was undetectable on oysters after 10 days, whereas on peppers, it was undetectable after only 1 day. HAV followed the same trend; HAV on oysters survived better by 1.4 to 4.1 log units than on peppers, depending on the temperature conditions. The exact location of inoculation could be an important factor. Viruses on peppers remained on the surface, while viruses were inoculated into the digestive tract of the oysters. These methods were used because viruses are generally exposed to the outer surfaces of fresh vegetables (42) and the digestive tract of shellfish (43-45). Harsh environmental conditions would be much more detrimental to viruses inoculated on vegetable surfaces than to those inside the digestive tract.

In addition, several research groups have proposed another reason as to why viruses can persist for long periods on oysters (46–48). With regard to the binding site of HuNoV, a certain carbohydrate complex known as histo-blood group antigen (HBGA) plays a major role as a virus receptor in humans and great apes (49–51). HBGAs are found widely within body components such as erythrocytes, saliva, and the surfaces of epithelial cells of various tissues. Other enteric viruses, such as Tulane virus, are also considered to use a type of HBGA as a binding receptor (52). Interestingly, an HBGA-like complex associated with binding has been verified to exist in oysters. Le Guyader et al. (47) found that HuNoV binds to oyster tissue through an A-like carbohydrate structure, which is also used for attachment to carbohydrate on human epithelial cells. Poliovirus (PV) was also shown to attach to shellfish mucus through ionic bonding, which is a similar process of viral attachment to host cells (53). Another previous study reported that hemocytes in oysters play an important role in the

TABLE 2 Summary of *D* values predicted by the biphasic model under different temperature and RH conditions

Virus	Temp (°C)	*D* value[a]			
		Oyster		Pepper	
		50% RH	70% RH	50% RH	70% RH
HAV	4	28.9	42.6	4.5	16.8
	15	15.8	11.3	0.7	11.4
	25	4.5	8.8	1.4	2.1
	40	0.8	0.8	0.3	0.1
MS2	4	47.7	15.9	17.2	13.7
	15	5.0	5.8	9.5	3.7
	25	1.5	8.5	9.1	1.7
	40	0.1	0.1	0.1	0.1
MNV	4	13.5	—	0.6	—
	15	2.8	—	0.4	—
	25	1.6	—	0.1	—
	40	0.1	—	0.0	—

[a] —, MNV was observed at 50% RH only.

retention of enteric viruses such as HAV, MNV, FCV, and PV (21). The ability of virus to tolerate low pH is required for survival within hemocytes in oysters. It is likely that specific binding or tolerance of acidic conditions may allow the virus to persist inside the digestive tissue.

In the case of vegetables, similar mechanisms were observed in previous studies. Virus-like particles (VLPs) of HuNoV attached to romaine lettuce (leaves and fresh-cut edges, etc.) by binding cell wall carbohydrates (e.g., GalNAc, GlcNAc, and sialic acid) (54). The surface charge of virions is associated with nonspecific binding to lettuce if viruses do not use specific cell surface receptors to attach (55). Both specific and nonspecific interactions between viruses and the surfaces of vegetables can affect binding ability. In light of this, enteric viruses may not perish rapidly on the surfaces of peppers because they bind to certain cell wall materials.

Water content in foodstuffs has been reported to play a significant role in stability and shelf life by influencing microbial growth (56, 57). During long-term storage, water activity reaches equilibrium with RH slowly, depending on factors such as the structure of food components, solvents, and other properties (58). This study was performed based on the assumption that the moisture content in virus-inoculated foods would be affected by the RH maintained in the chamber. However, an influence of RH on viral inactivation was not strongly observed in this study, especially in oysters. Survival of viruses on oysters decreased with rising temperatures but was rarely associated with variations in RH. Virus-inoculated oysters retained constantly high moisture contents, as they were stored in PBS. This means that the inactivation of viruses in oysters was solely the result of temperature. On the surface of peppers, the *D* value of HAV was lower in 50% RH, while the *D* value of MS2 was lower in 70% RH. The difference in *D* values was clearly greater at lower temperatures. Unlike oysters, peppers were exposed directly to both temperature and RH during the experiment. Therefore, we infer that virus survival was influenced mainly by temperature and that secondary effects occurred through the combination of temperature and RH. Previous studies have also described synergistic effects of temperature and humidity on viral inactivation (59, 60).

This study simultaneously compared the survival of HAV, MS2, and MNV on different food types. MNV and MS2, which are common surrogates of HuNoV, showed significantly different survival characteristics in this study ($P < 0.001$). At 4°C, the *D* value of MS2 was twice as high as that of MNV on oyster, and the *D* value MNV decreased much more rapidly than did that of MS2 on pepper. A recent review article (12) described extensive differences between several surrogates of HuNoV in susceptibility to temperature, environmental conditions, and chemical disinfectants. Therefore, not all virus surrogates are equal because of fundamental discrepancies, such as genome, host cell, and binding characteristics. In accordance with this, we suggest that the differences in inactivation rates between viral surrogates observed in this study might also be attributed to such biological factors.

In summary, our study demonstrated the prolonged survival of HAV, MS2, and MNV on typical foods without heat treatment. Our findings suggest that foodborne viruses may last for ~2 weeks at 4°C, which means that viruses can remain infectious under refrigerated conditions until foods are no longer consumable. This implies that the natural decay of viruses on foods might not provide adequate protection from viral pathogens once viral contamination occurs. Therefore, to prevent foodborne disease outbreaks, precautionary measures during food production and storage processes should be considered to be more efficient than efforts to reduce viral particles after contamination. This study will be useful information for the establishment of food safety guidelines.

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